B

types, including neurons, astrocytes, and oligodendrocytes (Reynolds and Weiss, 1992; Morshead et al., 1994; Weiss et al., 1996b). Recent data demonstrate that adult subventricular zone astrocytes or astrocyte progenitors can develop into stem cells in vivo (Doetsch, 1999).

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5.5 In vitro differentiation step of culturing cells

After the treatment step, the cells undergo an in vitro differentiation step that deprives the cells of the added factor used in the treatment steps. The in vitro differentiation step helps the cells transdifferentiate into various cell types including astrocytes, oligodendrocytes, and neurons. The cells may be cultured in various differentiation media that provide factors for cell survival. Suitable media includes a chemically defined medium such as DMEM/F12 supplemented with 1 µM RA, 1 mM dbcAMP and 30 ng per ml BDNF (Medium I); 30 ng per ml BDNF (Medium II); or 20 ng per ml GDNF, 10 ng per ml FGF-8, and 100 µM AA (Medium III). A medium supplemented with NEUROBASAL medium is also suitable (GIBCO). The cells are cultured on a substrate coated with laminin, poly-L-lysine, polyornithine, a suitable extracellular matrix factor, or the like.

In the Claims

Please cancel claims 2-3, 13-14, 17-22, 36-37, and 44-45 without prejudice or disclaimer. Please substitute the following amended claims for those currently pending:



1. (Once Amended) An in vitro method for producing neurons from astrocytes, the method comprising a culturing step of establishing a group of cells by culturing the astrocytes in vitro, and a subsequent treatment step of exposing the group of cells to at least one added factor that is a FGF family member such that neurons are produced as a result of the added factor.